Activation of toll-like receptor signaling influences expression of nociceptin and the nociceptin receptor in human NB4 cells

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Introduction

Nociceptin and its receptor (NOP) have been described as targets for the treatment of pain and inflammatory diseases.[1-3] However, mechanisms contributing to the regulation of the nociceptin system are still not fully understood. Toll-like receptors are a family of pattern recognition receptors which play a central role during inflammation. TLR activation has been reported to have an effect on opioid receptors and endogenous opioids.[4,5] The aim of this study was to investigate the effects of TLR signaling on the nociceptin system in human NB4 cells under inflammatory conditions.

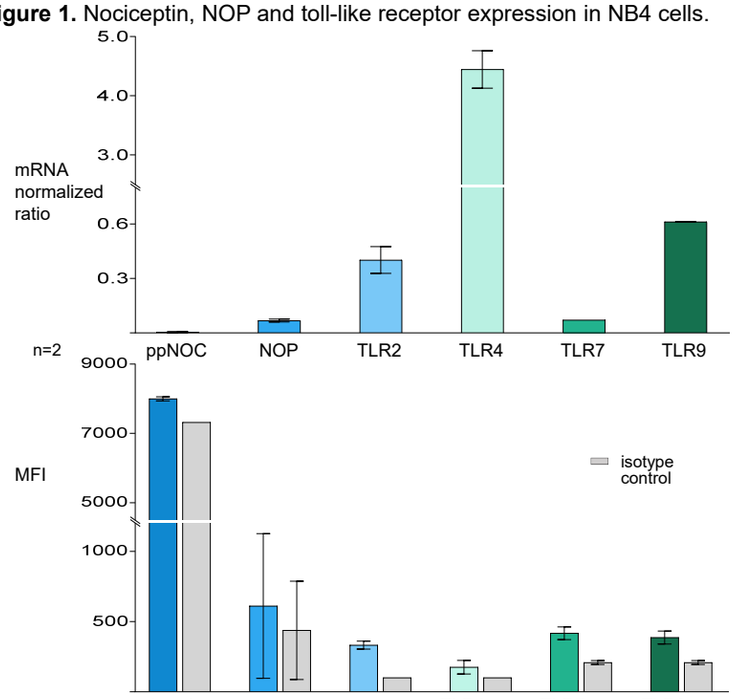
Methods

- Human promyelocytic leukemia NB4 cells were stimulated with or without different concentrations of phorbol-12-myristate-13-acetate (PMA).
- Quantitative RT-PCR: mRNA expression of prepronociceptin (ppNOC), NOP and TLRs (TLR2, TLR4, TLR7, TLR9).
- Flow cytometry: Cell surface NOP, TLR2, TLR4 and intracellular nociceptin, TLR7, TLR9 protein levels.
- To investigate effects of TLR activation on ppNOC and NOP mRNA expression, cells were cultured with or without PMA 5 ng/ml and with or without agonists specific for TLR2 (lipoteichoic acid, LTA 10 µg/ml), TLR4 (lipopolysaccharide, LPS 1 µg/ml), TLR7 (imiquimod, IMQ 10 µg/ml) or TLR9 (oligodeoxynucleotide [ODN] 2216 1 µM).
- Statistics: median, interquartile range (IQR); Kruskal-Wallis test, Wilcoxon signed-rank test, corrected for multiple testing; level of significance: p<0.05.

Results

Nociceptin, NOP and TLRs (TLR2, TLR4, TLR7, TLR9) mRNA were constitutively expressed and corresponding protein levels could be measured in NB4 cells (Figure 1).

Figure 1. Nociceptin, NOP and toll-like receptor expression in NB4 cells.



PMA 0.1-300 ng/ml dose-dependently upregulated ppNOC and NOP mRNA after 24 hrs. Based on these results, PMA 5 ng/ml was used in the subsequent experiments. PMA upregulated ppNOC, NOP, TLR4 and TLR9 mRNA expression and downregulated TLR2 mRNA after 24 hrs compared to controls (Figure 2).

The TLR7 agonist IMQ prevented PMA's upregulating effects on ppNOC and NOP mRNA expression. Cells stimulated with PMA and TLR9 agonist ODN 2216 showed an antagonistic effect on NOP mRNA compared to the samples treated with PMA only (Figure 3). As for nociceptin and NOP proteins, no changes were observed in NB4 cultured with PMA and different TLR agonists compared to the PMA-treated samples.

Figure 2. mRNA expression of ppNOC, NOP and TLRs in NB4 cells cultured with or without PMA 5 ng/ml for 24 hrs.

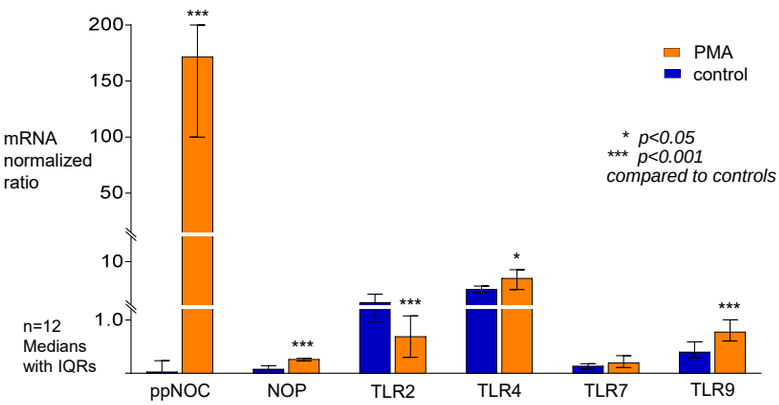
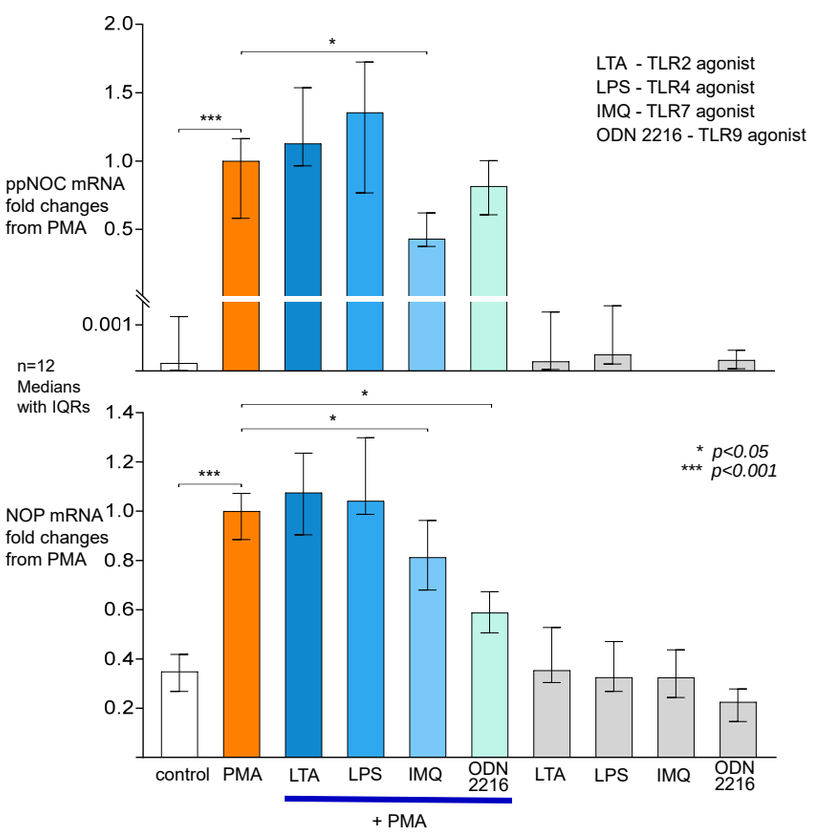


Figure 3. mRNA expression of ppNOC, NOP in NB4 cells cultured with or without PMA 5 ng/ml and with or without specific TLR agonists for 24 hrs.



Conclusions

- The present findings reveal additional details of the regulation of the nociceptin system and nociceptin's interaction with TLRs.
- Activation of TLR7 and TLR9 signaling downregulates mRNA expression of nociceptin and the nociceptin receptor in human NB4 cells under inflammatory conditions.
- Elucidating the regulation of nociceptin and its receptor and the mechanisms involved may lead to new insights into the treatment of pain and/or inflammation.

References

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